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Research Article

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Free radical scavenging activity of methanolic extract of brown alga *Spatoglossum asperum*

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ABSTRACT

The methanolic extract of Spatoglossum asperum showed radical scavenging activity against DPPH, ABTS, superoxide and nitric oxide radicals. Although the concentrations of methanolic extract of S.asperum. for antioxidant activity were much higher than that of standard drugs when tested in various assays, their maximal effects were approximately the same. For antioxidant activities, the methanolic extracts showed an increase with increasing concentration (between 100 and 900 µg/ml) indicating the dose dependency of these algal extract. The significant free radical scavenging activity of Spatoglossum asperum indicated that it could be a potential source of natural antioxidant lead molecules.

Keywords: *Spatoglossum asperum*, DPPH, ABTS, Superoxide radical, Nutraceutical.

INTRODUCTION

In recent years there is an upsurge in the areas related to newer developments in prevention of disease especially the role of free radicals and antioxidants. So it will be pertinent to examine the possible role of 'free radicals' in disease and 'antioxidants' in its prevention. Reactive oxygen species (ROS) (e.g., superoxide anion (O_2^-), hydroxyl radicals (OH•), hydrogen peroxide (H_2O_2), and singlet oxygen ($^1O_2$)) are formed as a result of normal metabolic activity and exogenous sources (Halliwell and Gutteridge, 1986). In pathological conditions, the antioxidant mechanisms are often inadequate, as excessive quantities of ROS can be generated. The ROS formed may cause cellular and sub cellular damage by peroxidation of membrane lipids, denaturing cellular proteins, breaking DNA strands and by disrupting cellular functions (Halliwell and Gutteridge, 1997). Over the last decade, considerable experimental evidence has confirmed the importance for health by following a diet rich in antioxidants, which can protect the organism against the damage caused by these radicals. Marine algae have received special attention as a source of natural antioxidants (Matsukawa *et al*., 1997). Seaweeds are known sources of pharmacological and food additives with potential health effects like antioxidative and anticarcinogenic (Lim *et al*., 2002; Athukurake *et al*., 2003).

EXPERIMENTAL SECTION

2.1 Collection of algal material

The marine brown alga *Spatoglossum asperum* was collected from the Mandapam, South East coast of Tamilnadu. The sample was identified by Emeritus Professor, Dr. R. Thevanathan, Professor in Botany, at CAS, University of Madras, Chennai.

2.2 Algal extracts preparation

The freshly collected samples were soaked and thoroughly cleaned in sea water to remove the sand and salt contents and shade dried. Dried seaweeds were powdered and soaked in methanol (1:3, w/v) overnight and filtered to collect the methanol fraction. The residue was extracted two more times and the filtrates were combined and concentrated to obtain the crude extract. All the fractions were concentrated by evaporating under vacuum in a rotary evaporator and the dried extract was used for exploring its potential activity.

2.3 Free radical scavenging activity

2.3.1 DPPH radical scavenging activity

DPPH radical scavenging activity was measured by the spectrophotometric method (Sreejayan and Rao, 1996). In this method methanolic extract of brown alga *S. asperum* was prepared in various concentrations from 100 to 900 µg/ml, each one of the above concentration was added to 0.05 ml of DPPH (200 µM). An equal amount of methanol was added to the control. After 20 mins, the absorbance was read at 517 nm and the percentage of inhibition was calculated by using the formula Prasanth *et al.,* (2000). The experiment was performed in triplicates.

$$\text{Percentage of Inhibition} = \frac{(\text{Control-test})}{\text{Control}} \times 100$$

2.3.2 1ABTS radical cation decolorisation assay

In this improved version of $ABTS^{\cdot}$, a free radical is generated by persulfate oxidation of 2, 2-azinobis (3-ethylbenzoline-6-sulfonic acid) - ($ABTS^{2-}$). ABTS radical cation was produced by reacting ABTS solution (7 mM) with 2.45 mM Ammonium PerSulphate and the mixture was allowed to stand in the dark at room temperature for 12-16 hrs before use. For the study, different concentrations (100 to 900 µg/ml) of methanolic extract (0.5 ml) were added to 0.3 ml of ABTS solution and the final volume was made up with ethanol to make 1ml. The absorbance was read at 745 nm and the percentage inhibition was calculated.

2.3.3 Scavenging of superoxide radical

Scavenging of superoxide radical was studied using the method elaborated by Winterbourn *et al*., (1975). Assay tubes contained 0.2 ml of the extract (corresponding to 20 mg extract) with 0.2 ml EDTA, 0.1 ml Nitro blue tetrazolium, 0.05 ml riboflavin and 2.64 ml phosphate buffer. The control tubes were set up with DMSO (Dimethyl sulfoxide) solution instead of the algal extracts. The initial optical densities of the solutions were recorded at 560 nm and the tubes were illuminated uniformly with the fluorescent lamp for 30 mins. A_{560} was measured again and the difference in O.D was taken as the quantum of superoxide production. The percentage of inhibition by the algal sample was calculated by comparing with O.D of the control tubes.

2.3.4 Scavenging of nitric oxide radical

Nitric oxide generated from sodium nitroprusside was measured by the Griess reaction (Sreejayan and Rao, 1996). Sodium nitroprusside (5 mM) in standard phosphate buffer solution was incubated with different concentration (100 to 900 µg/ml) of the methanol extract of *S. asperum* was dissolved in phosphate buffer (0.025 M, pH 7.4) and the solutions were incubated at 25^{o}C for 5 hrs. After 5 hrs, 0.5 ml of the incubated solution was removed and diluted with 0.5 ml of Griess' reagent (1% Sulphanilamide, 2% orthophosphoric acid and 0.1% naphthalene diaminedihydrochloride). The absorbance of the chromospheres formed during the diazotization of nitrite with sulphanilamide and its subsequent coupling with naphthalene diamine was read at 546 nm.

RESULTS AND DISCUSSION

In the present study, the free radical scavenging activity of methanolic extracts of *S.asperum* has been carried out by means of hydro soluble radicals (DPPH, ABTS, superoxide and nitric oxide radical).

An antioxidant is a compound which counteracts the effect of oxidized and controls the build of free radicals. Due to the presence of different antioxidant components in the crude extracts of biological tissue samples, it is relatively difficult to measure each antioxidant component separately. Therefore, several assay methods have been developed and applied in recent years to screen and evaluate the total antioxidant activity of such samples (Prabhakar *et al*., 2006; Wangensteen *et al*., 2004). These methods target at different mechanisms of the oxidant defense system such

as, scavenging active oxygen species and hydroxyl radicals, reduction of lipid peroxyl radicals, inhibition of lipid peroxidation, or chelation of metal ions.

Table. 1. Effect of methanolic extract of *Spatoglossum asperum* on different antioxidant models. (Free radical scavenging activity (inhibition %))

S.No	Concentration (µg/ml)	DPPH radical	ABTS radical	Superoxide radical	Nitric oxide radical
1	100	74.67 ± 0.01	58.93 ± 0.02	56.54 ± 0.03	68.25 ± 0.03
2	300	76.15 ± 0.03	78.84 ± 0.02	60.75 ± 0.02	73.15 ± 0.02
3	500	78.66 ± 0.02	89.96 ± 0.03	66.67 ± 0.02	75.75 ± 0.03
4	700	80.34 ± 0.03	92.95 ± 0.02	70.14 ± 0.03	76.82 ± 0.01
5	900	82.61 ± 0.02	96.15 ± 0.02	74.65 ± 0.02	80.29 ± 0.02
P - Value		0.000	0.000	0.000	0.000
F - Value		4.742444	1.109666	1.945555	7.641444

Mean ±SD

Table.2 IC_{50} values of experimental alga *S. asperum* and standard on free radical scavenging system. (Free radical scavenging assay IC_{50} value (µg/ml))

S.No	Experimental algae and Standard	DPPH radical	ABTS radical	Superoxide radical	Nitric oxide radical
1	*Spatoglossum asperum*	67	85	89	74
2	BHT	33	32.5	32	77.49
3	L-ascorbic acid	73	45.1	68.51	108.52

DPPH free radical scavenging activity

DPPH is stable free radicals that accept hydrogen to become a stable diamagnetic molecule. Hence DPPH is usually used as a substrate to evaluate the antioxidant activity (Elimastas *et al*., 2006). The results showed that *S.asperum* at a concentration of 900 µg/ml had the higher DPPH scavenging activity (82.61 ± 0.02%), but lesser than BHT (88.48 ± 0.18%) and L-ascorbic acid (92.46 ± 0.02%) (Tables.1). The IC_{50} value observed for *S. asperum* to be 67 µg/ml, it was also found that the IC_{50} value of the algal extracts was higher than that of both BHT 33 µg/ml and L-ascorbic acid 73 µg/ml (Table.2). The result is indicative of the hydrogen donating ability of and *S.asperum*, since the effects of antioxidants on DPPH radical scavenging is thought to be due to their hydrogen donating ability (Conforti *et al*., 2006). The results of the present study are in line with Wang *et al.,* (2009) and Yan *et al.,* (1999), who also found that brown algae contained higher amounts of polyphenols and DPPH radical scavenging activity than red and green algae. However, Chandini *et al.,* (2008) reported low levels of DPPH radical scavenging activity in brown seaweeds, in the range of 17.79 to 23.16% at an extract concentration of 1000 µg/ml.

ABTS radical scavenging activity

In the present study, *S.asperum* showed maximum % of inhibition (96.15 ± 0.02%), at 900 µg/ml concentration and these are slightly lower than that of the standard BHT (97.83 ± 0.18%) and L-ascorbic acid (97.32 ± 0.12%), (Tables.1). The IC_{50} values of $ABTS^{+}$ radical scavenging activity of methanolic extracts of experimental alga was 85 µg/ml and its IC_{50} values were higher than that of BHT (32.5 µg/ml) and L-ascorbic acid (45.1 µg/ml) (Table.2). The results of the present study indicate that the extract of brown seaweed exhibited higher ABTS radical activity. The results indicated that methanolic extract has a significant effect on scavenging of ABTS radicals. However, the limitations of ABTS assay, such as the capability of a sample to react with ABTS radical rather than to inhibit the oxidative process and the slow reaction of many phenolics (Roginsky and Lissi, 2005) necessitate compatible evaluation of antioxidant activity using other assays as well.

Superoxide anion scavenging activity

Superoxide scavenging activity of *S. asperum* exhibited a maximum of 74.65 ± 0.02% inhibition at the concentration of 900 µg/ml, which is equal with the standard BHT (74.23 ± 0.06%) and lower than L-ascorbic acid (78.58 ± 0.32%), (Tables.1). The IC_{50} values of BHT and ascorbic acid were shown in Tables2. The IC_{50} value of methanolic extracts of *S.asperum* was 89 µg/ml and it was higher than that of standard BHT (32 µg/ml) and L-ascorbic acid (68.51 µg/ml). The methanolic extracts of *Grateloupia lanceolata*, *Ahnfeltiopsis flabelliformis*, *Martensia denticulata*, *Bonnemaisonia hamifera*, *Carpopeltis affinis* and *Prionitis cornea a*re found to have relatively higher superoxide anion scavenging activities (over 83%). The results of the present investigation are in agreement with those of Le Tutour (1990) who investigated the antioxidant activities of different seaweeds in their studies and reported, *Laminaria digitata* and *Himanthalia elongata* exhibited the most valuable antioxidant activities

compared with those of vitamin-E and Butylhydroxy Toluene (BHT). Kuda *et al*., (2005) reported a good superoxide anion scavenging activity in edible brown seaweed, *Nemacystus decipiens*. Our study also exhibited strong superoxide anion inhibitory effect, in the methanolic extract of *S. asperum* and it can be used as an application in natural antioxidant source.

Nitric oxide free radical scavenging activity

The results showed that *S.asperum* had scavenging activity of 80.29 ± 0.02% and this value is comparably lower than that of the standard BHT (85.60 ± 0.40%) and L-ascorbic acid (90.51 ± 0.39%), (Tables.1). The IC_{50} values of the nitric oxide radical assay were compared to the standard antioxidants BHT (77.49 µg/ml) and L-ascorbic acid (108.52 µg/ml). The IC_{50} values of methanolic extracts of brown alga *S.asperum* was 74 µg/ml. It was also found that the IC_{50} value of the algal extracts was lower than that of L-ascorbic acid and BHT (Table.2). The suppression of nitric oxide release may be partially attributed to direct scavenging by the extracts of *S. asperum*, which decrease the amount of nitrite generated from the decomposition of sodium nitroprusside *in vitro* (Senevirathene *et al*., 2006). Scavengers of nitric oxide compete with oxygen leading to reduced production of nitric oxide (Green *et al*., 1982). The results of the present study are similar to Monsuang *et al*., (2009) who reported that brown seaweed *Sargassum* Sp, showed significantly higher phenolic content and antioxidant activities than red and green seaweeds. Recently, the seaweed extracts and fractions have been considered to be a rich source of antioxidants and different types of antioxidants have been isolated from various species of seaweeds (Fujimoto and Kaneda, 1984; Cahyana *et al*., 1992; Nagai and Yukimoto, 2003; Huang and Wang, 2004; Wang *et al*., 2009; Hu *et al*., 2010). The potential antioxidant compounds were identified as some pigments (fucoxanthin, astaxanthin, carotenoid e.g.) and polyphenols (phenolic acid, flavonoid, tannins e.g.), which are widely distributed in seaweeds and are known to exhibit higher antioxidative activities, which have been reported through various methods of reactive oxygen species scavenging activity and the inhibition of lipid peroxidation (Yan *et al*., 1999; Athukorala *et al*., 2003a; Athukorala *et al*., 2003b).

Seaweeds are known to contain reactive antioxidant molecules, such as ascorbate and glutathione (GSH) when fresh, as well as secondary metabolites, including carotenoids (α-and β-carotene, fucoxanthin, astaxanthin), mycosporine-like amino acids (mycosporine-glycine) and catechins (e.g., catechin, epigallocatechin, epigallocatechin), gallate, phlorotannins (e.g., phloroglucinol), eckol and tocopherols (α-, χ-, δ-tocopherols) (Yuan et al., 2005). Brown-algal polyphenols phlorotannins worked as antioxidants, antibacterial and anti-algal compounds (Kuda *et al*., 2007; Shibata *et al*., 2006). Based on the above facts, the marine brown alga *Spatoglossum asperum* was studied for its potential antioxidant property.

CONCLUSION

The results of the present work indicated that the methanolic extract of *S. asperum* was a fairly active scavenging assay system. The present findings seem promising to facilitate further experiments on the identification and characterization specific of compounds which are responsible for the relatively high antioxidant activities. Importantly, this research may contribute to a rational basis for the application of marine algal extract in possible therapy of diseases associated with oxidative stress and further supported that the antioxidant-rich extracts or fractions may be used as a dietary supplement, promoting good health.

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